**2161-Plat**

**A Novel Photosynthetic Strategy for Adaptation to Low Iron Environments**

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Iron availability is a major limiting factor for photosynthesis and hence for life in most of the aquatic environments on earth. . Cyanobacteria are important primary producers and prevail over Fe- deficiency by de-repressing the *isiAB* operon, which codes for the antenna protein IsiA and flavodoxin. We demonstrate that under nanomolar iron concentrations, a giant IsiA-Photosystem I supercomplex is formed, consisting of a Photosystem I trimer encircled by two complete IsiA rings with 18 and 25 copies in the inner ring and outer ring, respectively. The IsiA-Photosystem I supercomplex contains more than 850 chlorophylls and has a mass of 3.2 MDa, making it the most complex membrane protein that has been isolated to date. Ultrafast fluorescence spectroscopic results show fast and efficient excitation transfer and trapping in the supercomplex. The electron transfer throughput of Photosystem I is increased by 300%, an evolutionary adaptation that has allowed cyanobacteria to avoid oxidative stress. This adaptation of the photosynthetic apparatus confers an enormous, as-yet unrecognized, evolutionary advantage to cyanobacteria living under conditions of severe Fe stress and thereby have adapted to the modern low iron concentration in aquatic environments.

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# Platform AH: Membrane Receptors & Signal Transduction

**2162-Plat**

**A Novel Method to Probe Membrane Protein Topology Using Unnatural Amino Acid Mutagenesis and Antibody Epitope Tagging**

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We have developed a novel strategy to probe the topology of membrane proteins in their native bilayer environment. Our technique relies on unnatural amino acid mutagenesis to incorporate *p*-azido-L-phenlyalanine at a specific site in the expressed target protein. The reactive azido moiety facilitates Staudinger-Bertozzi ligation chemistry to introduce a monoclonal antibody (mAb) epitope-tagged phosphine derivative. This site-specific labeling method allows the flexibility and precision of single codon scanning, and appears to be superior to current biochemical approaches that rely on chemical modifications and/or introduction of epitope tags by mutagenesis. Our approach can be used to identify gross topological determinants for transmembrane proteins of unknown topology as well as to elucidate secondary structural elements with chemical precision. We demonstrate the experimental feasibility of our technique on human C-C chemokine receptor 5 (CCR5), a heptahelical transmembrane G protein-coupled receptor (GPCR) of known topology. CCR5 is a major co-receptor for HIV-1 entry into host cells. We labeled CCR5 in membranes with FLAG peptide epitope-phosphine at various sites on the receptor's intra- and extracellular surfaces. The differential reactivity of the FLAG epitope-phosphine reagent for the azido group on the various CCR5 mutants correlated to the known topology of CCR5 and defined specific helical boundaries. We further applied the new label/probe technology to mammalian cells in culture in order to label extracellular sites on surface-expressed CCR5. Our new method appears to be satisfactory to probe membrane protein topology in polytopic membrane proteins and has potential applications in the study of receptor signaling mechanisms in live cells.

**2163-Plat**

**Large-Scale MD Simulations Reveal Structural Elements of the Activated State in the 5-HT2a Receptor and their Relation to Cholesterol Dynamics**

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The 5-$HT_{2A}$ Serotonin receptor (5-$HT_{2A}$R) is a G-protein coupled receptor (GPCR) targeted by therapeutic drugs as well as hallucinogens such as LSD. We have previously shown that different modes of receptor activation and distinct cellular signaling are produced by hallucinogens and nonhallucinogenic congeners acting on the 5-$HT_{2A}$R (Weinstein, 2006). We characterize the ligand-dependent states of 5-$HT_{2A}$R from MD simulations of an experimentally-validated homology model based on rhodopsin and $\beta_2$AR structures complemented by cognate information about other GPCRs. At different stages of 0.3 μs simulations of 5-HT-bound 5-$HT_{2A}$R we observe sequential conformational changes that produce structural characteristics of receptor activation: (*1*) the extracellular part of TM6 moves inwards; (*2*) the toggle switch W6.48 flips to become parallel to the membrane; (*3*) the intracellular part of TM6 moves outwards and (*4*) the ionic lock in the DRY motif breaks. In the parallel simulations of 5-$HT_{2A}$R with LSD (currently at 0.12 μs), only some of the same active-like components are observed (e.g., TM7 moving away from TM2, and the breaking of the ionic lock). Comparing the dynamics of 5-$HT_{2A}$R complexed with LSD and 5-HT, we found that H8 of the LSD-bound receptor undergoes less rearrangement during the same time period, and that the interaction of extracellular loop 2 with LSD is stronger than with 5-HT, but that local distortions in TMs (e.g., proline kinks) are similar in the two systems. Interestingly, correlation analysis indicates that some of the local changes in 5-$HT_{2A}$R relate to the dynamics of cholesterol in the membrane, at a series of preferred sites of interaction with the receptor, in a manner similar to those reported previously from μs rhodopsin simulations (Grossfield et al., 2006; Khelashvili et al., 2009).

**2164-Plat**

**Molecular Model of the Opsin-G-Protein Complex**

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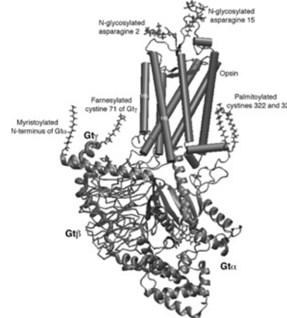
A recent highlight in the structural biology of G-protein-coupled receptors includes the structural elucidation of opsin in an active-state conformation (Ops*). We modeled the complex of opsin with the heterotrimeric G-protein transducin (Ops*-$G_t\alpha\beta\gamma$, figure below), using the NMR structure of an 11-residues peptide from the C-terminus of the alpha-subunit of transducin ($G_t\alpha$CT) and the crystal structures of opsin in its G-protein interacting conformation (Ops*-$G_t\alpha CT_{K341L}$), of rhodopsin in an inactive state, and of transducin ($G_t\alpha\beta\gamma\bullet$GDP). We reconstructed the C-terminal α5 helix of $G_t\alpha$ and docked it to the open binding site in Ops*. It has been proposed that a 40°-tilt of $G_t\alpha\beta\gamma$ relative to the α5 helix is necessary to avoid steric clashes between $G_t\beta\gamma$ and the membrane. We propose an alternative model without these massive changes in α5 helix packing. With an alternative conformation of the intra-cellular loop connecting helix 5 and helix 6 in Ops* it is possible to obtain a model that maintains the Ops*-induced $\alpha_L$-type C-capping conformation of $Gt_\alpha$CT and its key interactions with Ops*. The model will be used for detailed MD simulations of the complex in the membrane and to design cross-linking experiments for biochemical validation.




**2165-Plat**

**Insights into G-Protein Coupled Receptors Activation from All-Atom Molecular Dynamics Simulations**

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G protein coupled receptors (GPCRs) are a large family of integral membrane proteins involved in many signal transduction pathways. The recently crystallized structures of two engineered adrenergic receptors (ARs)[1,2] and of ligand-free opsin bound to a G-protein peptide[3] have opened new avenues for the understanding of the molecular mechanisms of action of GPCRs, but they also generated some controversy on the proposed mechanism of GPCR activation.

To understand the molecular details of GPCR activation, we carried out submicrosecond molecular dynamics simulations of wild type β1AR and β2AR and of rhodopsin dimer in explicit lipid bilayer under physiological conditions.

Our simulations showed that the equilibrated structures of ARs recover all the previously suggested features of inactive GPCRs, including formation of a crucial salt bridge between the cytoplasmatic moieties of helices III and VI (''ionic-lock'') that is absent in the crystal structures[4].

We found that cooperation between a number of highly conserved residues is a key component in the early steps of the activation mechanism of diffusible